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Proposed Registration Decision PRD2013-03

Metarhizium anisopliae strain F52

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Overview

Proposed Registration Decision for *Metarhizium anisopliae* Strain F52

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide, containing the technical grade active ingredient *Metarhizium anisopliae* strain F52, to reduce the numbers of whiteflies and thrips on greenhouse food crops and suppress hairy chinch bugs and ticks on turf.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment. This is a major new use for this active ingredient.

This Overview describes the key points of the evaluation, while the Science Evaluation Section provides detailed technical information on the human health, environmental and value assessments of *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (such as children) as well as organisms in the environment (those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Metarhizium anisopliae* strain F52, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Metarhizium anisopliae* strain F52, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation Section of this consultation document.

What Is *Metarhizium anisopliae* Strain F52?

Metarhizium anisopliae strain F52 is a soil-dwelling fungus which infects insects and ticks and can result in death. Formulated as Met52 EC Bioinsecticide and applied as a foliar spray it reduces whitefly numbers on greenhouse tomato, reduces thrips numbers on greenhouse pepper, greenhouse strawberry and greenhouse zucchini, and suppresses hairy chinch bug and ticks on turf. A granular formulation of *Metarhizium anisopliae* strain F52 (Met52 Granular Bioinsecticide) is currently registered for the control of black vine weevil and strawberry root weevil when applied in the growth media of container grown ornamentals. Both end-use products are Commercial Class.

Health Considerations

Can Approved Uses of *Metarhizium anisopliae* strain F52 Affect Human Health?

***Metarhizium anisopliae* strain F52 is unlikely to affect your health when Met52 EC Bioinsecticide is used according to the label directions.**

People could be exposed to *Metarhizium anisopliae* strain F52 from the application Met52 EC Bioinsecticide. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *Metarhizium anisopliae* strain F52 were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

When *Metarhizium anisopliae* strain F52 was administered orally to rats, no signs of toxicity or disease were observed. Although *Metarhizium anisopliae* strain F52 can produce toxic metabolites, analytical data were submitted showing that these were not detected in the technical grade active ingredient. Furthermore, the growth of *Metarhizium anisopliae* strain F52 on insect hosts is unlikely to result in residues of metabolic byproducts occurring on food commodities. Therefore, the establishment of an MRL is not required for *Metarhizium anisopliae* strain F52. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Met52 EC Bioinsecticide

Occupational risks are not of concern when Met52 EC Bioinsecticide is used according to label directions, which include protective measures

Occupational risks are not of concern when Met52 EC Bioinsecticide is used according to label directions, which include protective measures.

Workers handling Met52 EC Bioinsecticide can come into direct contact with *Metarhizium anisopliae* strain F52 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product must wear waterproof gloves, long-sleeved shirts, long pants, eye goggles, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Met52 EC Bioinsecticide Is Introduced Into the Environment?

Environmental risks are not of concern

Metarhizium anisopliae strain F52 is a non-indigenous soil microorganism that is pathogenic to specific host insects. Since the reproduction of conidiospores is dependent upon infection of a suitable host under conditions of high humidity, the proliferation of *Metarhizium anisopliae* strain F52 in the environment would be limited. It is likely that levels of *Metarhizium anisopliae* strain F52 would return to levels comparable to native populations of *Metarhizium anisopliae* after application of Met52 EC Bioinsecticide.

Toxicity testing on non-target organisms shows that *Metarhizium anisopliae* strain F52 is capable of causing some adverse effects to certain aquatic organisms when exposed to high concentrations. However, the end-use product label includes instructions for applicators to control spray drift, therefore, the application of Met52 EC Bioinsecticide onto turfgrass and vegetable crops in greenhouses is unlikely to result in significant contamination of aquatic environments. Therefore, the risk to aquatic organisms from the use of Met52 EC Bioinsecticide is low. Toxicity testing also shows that terrestrial non-target organisms, other than target insect species, were not adversely affected by *Metarhizium anisopliae* strain F52 when exposed to high concentrations.

Value Considerations

What Is the Value of Met52 EC Bioinsecticide?

Applied as a foliar spray, Met52 EC Bioinsecticide reduces whitefly numbers on greenhouse tomato, reduces thrip numbers on greenhouse pepper, greenhouse strawberry and greenhouse zucchini, and suppresses hairy chinch bug and ticks on turf.

Met52 EC Bioinsecticide is for use against whiteflies and thrips on greenhouse food crops, hairy chinch bugs and ticks on turf. Whiteflies and thrips are serious pests of greenhouse food crops. Chinch bugs can be serious pests of turf grass. Most pest control products for these uses are conventional chemical insecticides. Few pest control products are registered for use against ticks in turf.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Met52 EC Bioinsecticide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed to large quantities of Met52 EC Bioinsecticide, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since the end-use product has been identified as a sensitizer. Therefore, anyone handling or applying Met52 EC Bioinsecticide must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Due to the irritation potential identified for Met52 EC Bioinsecticide, workers and handlers are also required to wear eye goggles. Also, the signal words, "POTENTIAL SENSITIZER", "WARNING – EYE IRRITANT" and "CAUTION – SKIN IRRITANT" must appear on the principal display panel and precautionary statements, "DO NOT get in eyes. Avoid contact with skin." and "May cause sensitization." are required on the secondary display panel of the label.

Environment

The end-use product label will include standard environmental precaution statements that prevent the contamination of aquatic systems from the use of Met52 EC Bioinsecticide.

Next Steps

Before making a final registration decision on *Metarhizium anisopliae* strain F52, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Metarhizium anisopliae* strain F52 (based on the Science Evaluation section of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Metarhizium anisopliae strain F52

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Metarhizium anisopliae* strain F52
Function	To reduce the number of whiteflies on greenhouse tomato; reduce the number of thrips on greenhouse pepper, strawberry and zucchini; and suppress hairy chinch bugs and ticks on turf.
Binomial name	*Metarhizium anisopliae* var. *anisopliae* strain F52
Taxonomic designation[1]	
Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Subphylum	Pezizomycotina
Order	Hypocreales
Family	Clavicipitaceae
Genus	*Metarhizum*
Species	*Anisopliae*
strain	F52
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	Technical grade active Ingredient (TGAI): 1.0×10^{10} colony forming units (CFU)/g End-use Product: 2.0×10^{9} CFU/mL
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Metarhizium anisopliae* strain F52 is known to produce cytochalasin and destruxin, which are secondary metabolites of concern (see Section 3.4.1).

[1] Taxonomy browser at: http://www.ncbi.nlm.nih.gov/pubmed/

1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-Use Product

Technical Grade Active Ingredient: *Metarhizium anisopliae* strain F52, and End-Use Product: Met52 EC Bioinsecticide

Properties	*Metarhizium anisopliae* strain F52	Met52 EC Bioinsecticide
Physical state	Powder	Liquid
Colour	Grayish-olive	Green; greenish-black; very dark brown; almost black
Odour	Earthy	Faint or no-odour
pH (1% w/v)	5.66 in 1% (w/v) reagent water	6.16 at 1% (w/v) in distilled water
Bulk Density	0.273 g/mL (bulk)	0.89 g/mL
Guarantee	1.0×10^{10} CFU/g	2.0×10^{9} CFU/mL

1.3 Directions for Use

The approved application rates are shown in the table below. Spray to wet all foliage, but avoid runoff. Make the first Met52 EC Bioinsecticide application against low population levels. Use the higher application rate when pest pressure is high. Re-apply as required. The need for, and the timing of re-application should be determined by monitoring. For greenhouse crops, the re-application interval is 5–10 day. The re-application intervals for turf are four weeks for hairy chinch bugs and three weeks for ticks. The active ingredient of Met52 EC Bioinsecticide is live fungus spores. The product must not be tank mixed with fungicides or applied with thermal foggers. Fungicides should not be applied near the time of Met52 EC Bioinsecticide applications.

Met52 EC Bioinsecticide application rates

Crop	Pest	Concentration or Rate
Greenhouse Tomato	Whiteflies (reduces numbers)	0.5–5.0 L/1000 L
Greenhouse Pepper, Greenhouse Strawberry, Greenhouse Zucchini	Thrips (reduces numbers)	0.5–5.0 L/1000 L
Turf	Hairy chinch bug (suppression)	64–96 mL/100 m^2
	Ticks (suppression)	137–155 mL/100 m^2

1.4 Mode of Action

Met52 EC Bioinsecticide is composed of spores of the entomopathogenic fungus *Metarhizium anisopliae* strain F52. When the product is applied to the foliage, immature and adult insects or ticks that come into contact with the spores become infected. Under ideal conditions death may occur within 3–7 days after exposure to the spores.

The level of control exerted by Met52 EC Bioinsecticide can be inconsistent. This is often the case for fungal pathogens of insects and ticks because the rate of infection depends on other factors in addition to the number of spores per surface area of the crop. Infection rate depends on the susceptibility of individual insects/ticks and the environmental conditions that effect spore survival such as relative humidity, temperature and light frequency and intensity.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Metarhizium anisopliae strain F52 is not substantially different from the classical description of the species. The dominant taxonomic characteristics are the morphological features of the sporulating structures. The genus is defined on the basis of the arrangement of the phialides-bearing chains and columns of dry and generally green, cylindrical or slightly ovoid conidia. The columns are formed by aggregation of the conidial chains. There are two distinguished forms of *Metarhizium anisopliae* based on the conidial size: (1) the short-spored form *Metarhizium anisopliae* var. *anisopliae*, with conidia of about 5–8 µm long and (2) the long-spored form *Metarhizium anisopliae* var. *majus*, with conidia usually between 10 and 14 µm long. A method has been developed to differentiate between strains of *Metarhizium anisopliae* var. *anisopliae* by the identification of group-I introns at three different positions within the 28S rDNA gene of the *Metarhizium anisopliae* var. *anisopliae*, although, this does not appear to be a routine component of the quality assurance program.

2.2 Methods for Establishment of Purity of Seed Stock

Subsamples of the mother culture are frozen in a large number of aliquots at -80°C in 15% glycerol. These aliquots are in sufficient numbers to provide starter cultures for several years of production. Once these starter cultures have been depleted, bioassays are used to determine if passage through an insect target host such as black vine weevil is necessary to retain pathogenicity. *Metarhizium anisopliae* strain F52 is also deposited in three culture collections; one held by the Centre for Agriculture and Biosciences International (IMI 385045), a second held by the Agricultural Research Service Entomopathogenic Fungi (ARSEF 7711) and a third by the American Type Culture Collection (ATCC 90448).

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee of the end-use product is based on the number of viable spores per volume of product. The total spore count, determined by means of a hemacytometer, is multiplied by the germination rate, determined by microscopic examination of culture plates for the development of germ tubes, to give a guarantee measured in CFU/mL of end-use product.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted in Section 2.1, the microbial pest control agent (MPCA) can be identified to the species variant (*Metarhizium anisopliae* var. *anisopliae*) level by the identification of group-I introns at three different positions within the 28S rDNA gene. These methods can help to identify strain F52; however, these methods alone may not be sufficient to distinguish this strain of *Metarhizium anisopliae* var. *anisopliae* from all other naturally occurring strains. Although secondary metabolites of toxicological significance (destruxins and cytochalasins) have been shown to be produced by *Metarhizium. anisopliae* strain F52, analytical data were submitted showing that these metabolites were below the limit of detection (LOD) in the TGAI. Furthermore, *Metarhizium anisopliae* strain F52 is only expected to grow on the host insect and the secondary metabolites in question are expected to decompose quickly following host insect death. It is also unlikely that these metabolites would translocate to edible portions of crops due to the detoxification mechanisms found in plants. Furthermore, when spores of *Metarhizium anisopliae* strain F52 were administered orally to rats, no signs of toxicity or disease were observed. Therefore, no methods are required to quantify viable or non-viable residues of *Metarhizium anisopliae* strain F52.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide are acceptable.

During manufacturing, several approaches will be used to limit microbial contamination in the TGAI and end-use product. These approaches will include sterilization of media and visual inspection of growth cultures for contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of four production batches of end-use product using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use product does not contain unacceptable levels of human and animal disease-causing microorganisms.

The secondary metabolites cytochalasins and destruxins were not present in the end-use product at the LOD.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing of two batches of the end-use product showed that these products are stable when stored at 4°C for a period of up to nine months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

Please refer to the previously published summary under Section 3.1, Toxicity and Infectivity Summary, of the published document Evaluation Report ERC2010-01, *Metarhizium anisopliae* strain F52. Below is a summary of data provided to support the new end-use product, Met52 EC Bioinsecticide.

Two high-performance liquid chromatography studies were submitted examining extracts from two cultures of *Metarhizium anisopliae* strain F52 for the content of destruxins (A, B, CHL, and E diol) and cytochalasins (A, B, C, D, E, and J). The methods used in both studies were, for practical purposes, identical. One culture used a nutrient rich liquid media (Sabouraud Dextrose Broth with 1% yeast extract) and the other culture used a media that was similar or identical to the media used to manufacture the MPCA and end-use product. In the study examining extracts from nutrient rich liquid media, it was confirmed that *Metarhizium anisopliae* strain F52 will produce cytochalasins and destruxins under optimal conditions of growth. In the study examining extracts from the growth media used in the production of the MPCA, it was shown that cytochalasins and destruxins may have been produced, but they only occurred at levels below the LOD if they were present at all.

In an acute dermal toxicity study, groups of 14-week-old New Zealand White rabbits [5/sex] were dermally exposed to TAE-001 Oil (equivalent to Met52 EC Bioinsecticide) for 24 hours to an area of skin approximately 10% of the total dorsal body surface area. Following exposure, the animals were observed for a period of 14 days. The dermal LD_{50} was >5g/kg bodyweight (male and female). TAE-001 Oil is of low toxicity based on the dermal LD_{50}. There were no treatment related clinical signs, necropsy findings or changes in body weight. There were signs of dermal irritation observed (erythema and/or edema; cracking of the skin; eschar formation; and flaking of the skin). This acute dermal toxicity study is classified as acceptable.

In a primary dermal irritation study, young adult New Zealand White rabbits (2♂; 1♀) were dermally exposed to 0.5 mL of TAE-001 Oil (equivalent to Met52 EC Bioinsecticide) for four hours. Animals were then observed for seven days. Irritation was scored by the method of Draize. Very slight to well-defined erythema and edema was observed in the two male rabbits. Superficial flaking of the test site skin was observed in the two male rabbits on Day 7, otherwise, all rabbits had completely recovered from signs of derma irritation by that time. In this study, TAE-001 Oil is slightly irritating to the skin based on a Maximum Irritation Score of 1.33 at 72 h. It is recommended that the end-use product label contain the following statements:

Primary Display Panel: 'CAUTION – SKIN IRRITANT'
Secondary Display Panel: 'May irritate the skin. Avoid contact with skin.'

3.2 Dietary Exposure and Risk Assessment

3.2.1 Food

While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *Metarhizium anisopliae* strain F52 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Although secondary metabolites of toxicological significance (destruxins and cytochalasins) have been shown to be produced by *Metarhizium anisopliae* strain F52, analytical data were submitted showing that these metabolites were below the LOD in the TGAI. Furthermore, *Metarhizium anisopliae* strain F52 is only expected to grow on the host insect and the secondary metabolites in question are expected to decompose quickly following host insect death. It is also unlikely that these metabolites would translocate to edible portions of crops due to the detoxification mechanisms found in plants. Considering these facts regarding the secondary metabolites of toxicological concern and due to the demonstrated low-toxicity of the MPCA, the risks from secondary metabolites to the general population, including infants and children, or animals are negligible. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.2.2 Drinking Water

The likelihood of *Metarhizium anisopliae* strain F52 entering neighbouring aquatic environments or surface water run-off from greenhouse or field use of Met52 EC Bioinsecticide is considered very low.

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The end-use product label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent from greenhouses or runoff containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water. Therefore, potential exposure to *Metarhizium anisopliae* strain F52 in surface and drinking water is negligible.

3.2.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general

assessment of risk if no significant adverse effects (no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that *Metarhizium anisopliae* strain F52 is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCAs, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCAs and other registered micro-organisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *Metarhizium anisopliae* strain F52 to human health.

3.3 Maximum Residue Limits

Residues of *Metarhizium anisopliae* strain F52 on treated food crops, at the time of harvest, are anticipated. Consequently, the Agency has applied a hazard-based approach for determining whether an MRL is required for this microorganism. Based on the lack of toxicity and pathogenicity effects observed in the acute toxicity and infectivity studies (particularly the oral study) and the fact that although *Metarhizium anisopliae* strain F52 is known to produce secondary metabolites of toxicological significance (destruxins and cytochalasins), the risks anticipated for dietary exposure are considered low since analytical data were submitted showing that these metabolites were below the LOD in the TGAI; *Metarhizium anisopliae* strain F52 is only expected to grow on the host insect; and the secondary metabolites in question are expected to decompose quickly following host insect death; that it is unlikely that these metabolites would translocate to edible portions of crops due to the detoxification mechanisms found in plants, the risks anticipated for dietary exposure are considered low. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that an MRL does not need to be established for *Metarhizium anisopliae* strain F52.

Metarhizium anisopliae strain F52 is exempt from the requirement of a food tolerance in the United States.

3.4 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the Agency's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *Metarhizium anisopliae* strain F52 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since re-entry to into treated areas (turf, residential and recreational) is to be restricted until spray has dried. Furthermore, few adverse effects from exposure to *Metarhizium anisopliae* encountered in the environment have been

reported. Even if there is an increase in exposure to this microorganism from the use of Met52 EC Bioinsecticide, there should not be any increase in potential human health risk.

3.5 Cumulative Effects

The Agency has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *Metarhizium anisopliae* in the environment, the Agency is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *Metarhizium anisopliae* strain F52. No cumulative effects are anticipated if the residues of *Metarhizium anisopliae* strain F52 interact with related strains of this microbial species.

3.6 Occupational / Bystander Exposure and Risk Assessment

3.6.1 Occupational

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Metarhizium anisopliae* has not been identified as a dermal wound pathogen, and there is no indication that it could penetrate intact skin of healthy individuals and does not contain any toxic secondary metabolite that could be dermally absorbed. Furthermore, dermal toxicity studies in animals demonstrated no signs of systemic toxicity to *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide.

The toxicity testing with the *Metarhizium anisopliae* strain F52 showed no toxicity or infectivity via the oral, dermal, or pulmonary routes of exposure. The submitted dermal toxicity and irritation study with the end-use product demonstrated a potential for skin irritation, and submitted eye irritation study with the TGAI indicates that *Metarhizium anisopliae* strain F52 is irritating to eye; therefore, precautionary label statements to avoid contact of the end-use product with skin and eyes, and use of proper clothing and personal protective equipment, such as waterproof gloves and protective eye-wear are required to mitigate occupational exposure concerns.

Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed end-use product use, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Met52 EC Bioinsecticide, and no significant occupational risks are anticipated from this product.

3.6.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed by commercial applicators in the use of Met52 EC Bioinsecticide.

Although the label does allow applications to turf, residential or recreational areas; dermal and inhalation exposure to the general public will be very low since re-entry to into treated areas (turf, residential and recreational) is to be restricted until spray has dried. Therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the re-entry to treated sites is to be restricted until the product has dried, exposure to infants and children in school, residential and day care facilities is likely to be minimal. Consequently, no adverse effects are anticipated due to the minimal exposure to bystanders and because *Metarhizium anisopliae* strain F52 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study.

3.7 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA. Information on the reporting of incidents can be found on the PMRA website. Incidents were searched and reviewed for the active ingredient *Metarhizium anisopliae* strain F52. As of 4 January 2013, there were two incidents, involving seven people, reported to the PMRA for *Metarhizium anisopliae* strain F52. All incidents occurred in a plant that manufactures the TGAI. There were six cases that were classified as minor and two as moderate in severity.

Overall, there was a good degree of association between the reported exposure and the symptoms, with all incidents considered to be at least possibly related to the reported exposure. The incidents all involved respiratory exposure. Symptoms were respiratory, dermal and general in nature.

These incident reports were considered in this evaluation and did not affect the risk assessment.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether an MPCA is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent of exposure. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only

triggered if significant toxicological effects in non-target organisms are noted in Tier I testing (see Section 4.2 for additional details on the various Tier levels).

4.2 Effects on Non-Target Species

The PMRA has a four-level tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or maximum challenge concentration of the MPCA. The maximum challenge concentration is generally derived from the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate multiplied by some safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (that is, life cycle studies) as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on Terrestrial Organisms

A complete ecotoxicology package was submitted and reviewed to address the risks of *Metarhizium anisopliae* strain F52 to terrestrial organisms published under Evaluation Report ERC2010-01, *Metarhizium anisopliae* strain F52.

In that review, it was noted that pathogenicity was not adequately assessed in terrestrial organisms. This was because the viability of the test material used in the terrestrial non-target organism testing was not confirmed in the original review, Evaluation Report ERC2010-01, *Metarhizium anisopliae* strain F52. The applicant has now submitted certificates of analysis of the test substances used in the studies verifying the viability. With this new information it can be stated that pathogenicity has been assessed in the non-target terrestrial organism studies and that *Metarhizium anisopliae* strain F52 showed no signs of pathogenicity in any of the tested birds, terrestrial arthropods, honeybees, or earthworms.

Based on all the available data and information on the effects of *Metarhizium anisopliae* strain F52 to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target terrestrial microorganisms from the use of Met52 EC Bioinsecticide on turf or in greenhouses.

4.2.2 Effects on Aquatic Organisms

A complete ecotoxicology package was submitted and reviewed to address the risks of *Metarhizium anisopliae* strain F52 to aquatic organisms published under Evaluation Report ERC2010-01, *Metarhizium anisopliae* strain F52.

In that review, it was noted that pathogenicity was not adequately assessed in fish, daphnids, and algae. This was because the viability of the test material used in the aquatic non-target organism testing was not confirmed in the original review, Evaluation Report ERC2010-01, *Metarhizium anisopliae* strain F52. The applicant has now submitted certificates of analysis of the test substances used in the studies verifying the viability. With this new information it can be stated that pathogenicity has been assessed in fish, aquatic arthropods and aquatic plants and that *Metarhizium anisopliae* strain F52 showed no signs of pathogenicity in any of these aquatic non-target organisms.

Based on all the available data and information on the effects of *Metarhizium anisopliae* strain F52 to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the use of Met52 EC Bioinsecticide on turf or in greenhouses. Although there is evidence that *Metarhizium anisopliae* strain F52 is capable of adversely affecting certain aquatic non-target organisms, the likelihood of such an event occurring is low since the end-use product label includes standard environmental precaution statements to prevent the contamination of aquatic habitats. The screening level risk assessment was conducted based on the estimated environmental concentration for the highest use rate scenario for *Metarhizium anisopliae* strain F52 in Met52 EC Bioinsecticide on turf (15.5 L/ha or 3.1×10^{13} CFU/ha). The level of concern was not exceeded for daphnids.

4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-

proteger/incident/indexeng.php. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

As of 3 January 2012, there were no environmental incidents reported in the PMRA Incident reporting database or in the United State Environmental Protection Agency's Ecological Incident Information System for products containing *Metarhizium anisopliae* for use as pesticides.

5.0 Value

5.1 Effectiveness Against Pests

Submitted efficacy data demonstrated that Met52 EC Bioinsecticide infects whiteflies on the foliage of greenhouse tomatoes (nine trials), as well as thrips on the foliage of greenhouse peppers, strawberries and zucchini (nine trials). Trials tested multiple applications at 0.5, 2.5 and 5.0 L product/1000 L water. Assessments were made after each application. The reduction of whitefly numbers was low and inconsistent. The reduction of thrips numbers was higher than for whiteflies but still inconsistent. A treatment effect of Met52 EC Bioinsecticide was evident for 5-10 days against whiteflies and thrips on greenhouse food crops.

The application rate range against hairy chinch bugs on turf is 64-96 mL product/100 m^2. Efficacy trials demonstrated that a single application at 32, 64 and 67 mL/100 m^2 suppressed hairy chinch bugs; however the levels of control were not consistent. Although the high rate (96 mL product/100 m^2) was not tested, Met52 EC Bioinsecticide is a biological pesticide and its efficacy depends on many factors such as humidity and temperature. Therefore, to give users flexibility, 64–96 mL/100 m^2 was accepted. The effect of Met52 EC Bioinsecticide lasted four weeks against hairy chinch bugs on turf.

Efficacy trials demonstrated that all Met52 EC Bioinsecticide rates tested, 137, 155 and 619 mL Met52 EC Bioinsecticide/100 m^2, were equally effective at suppressing ticks. Therefore, to give users flexibility, a label claim of suppresses ticks at 137–155 mL product/100 m^2 was accepted. The effect of Met52 EC Bioinsecticide lasted three weeks against ticks on turf.

5.1.1 Acceptable Efficacy Claims

Met52 EC Bioinsecticide reduces whitefly numbers on greenhouse tomato and reduces thrips numbers on greenhouse pepper, greenhouse strawberry and greenhouse zucchini at 0.5–5.0 L product/1000 L water with a recommended 5–10 day application interval. For both pests, application of the product will reduce numbers, but the reduction may not be substantial. Met52 EC Bioinsecticide suppresses hairy chinch bug on turf at 64–96 mL product/100 m^2 and ticks on turf at 137–155 mL product/100 m^2. Application intervals of three weeks for ticks or four weeks for hairy chinch bugs are recommended. The need for and the timing of re-application should be determined by monitoring. The higher application rate is to be used when pest pressure is high.

Aerial application to turf is not supported since aerial application is not applicable to turf uses in Canada.

5.2 Phytotoxicity to Host Plants

Efficacy trials conducted on greenhouse plants reported no adverse effects on the host plants. The turf trials did not mention phytotoxicity.

5.3 Sustainability

5.3.1 Survey of Alternatives

The majority of the alternatives to Met52 EC Bioinsecticide uses are conventional active ingredients (Appendix I, Table 2). Non-conventional pesticide active ingredient alternatives are insecticidal soap and another entomopathogenic fungus, *Beauveria bassiana* strain GHA.

5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

Met52 EC Bioinsecticide is generally compatible with management practices for greenhouse food crops and turf. Fungicides should not be tank mixed with Met52 EC Bioinsecticide or applied near the time of application of Met52 EC Bioinsecticide. Compatibility of Met52 EC Bioinsecticide with greenhouse or turf biological control agents has not yet been established.

5.3.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

The development of resistance to entomopathogenic fungi has not been documented and, due to their mode of action, is not considered likely.

5.3.4 Contribution to Risk Reduction and Sustainability

Met52 EC Bioinsecticide may contribute to resistance management in integrated pest management programs for whiteflies and thrips on labelled greenhouse food crops and for hairy chinch bug and ticks on turf.

6.0 Pest Control Product Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy: persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity, and toxic as defined by the *Canadian Environmental Protection Act*].

During the review process, Met52 EC Bioinsecticide and *Metarhizium anisopliae* strain F52 were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track I criteria. The PMRA has reached the following conclusions:

- *Metarhizium anisopliae* strain F52 does not meet the Track 1 criteria because the active ingredient is an organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.1 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy (TSMP)*, and DIR2006-02, *Formulants Policy and Implementation Guidance Document*[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Met52 EC Bioinsecticide and *Metarhizium anisopliae* strain F52 do not contain any other formulants or contaminants of environmental concern identified in the *Canada Gazette*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

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5 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*

6 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

7 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*

8 Regulatory Directive DIR2006-02, *PMRA Formulants Policy*

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide were deemed adequate to assess their potential human health and environmental risks. The end-use product was supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of 9 months when stored at 4°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information previously reviewed in ERC2010-01, *Metarhizium anisopliae* strain F52 as well as the additional acute dermal toxicity and dermal irritation data provided on the end-use product were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests, spores of *Metarhizium anisopliae* strain F52 were of low toxicity by the oral, pulmonary and dermal routes, and were not pathogenic or infective via the oral, intraperitoneal injection and pulmonary exposure routes in animals. The TGAI and the end-use product have the potential to irritate eyes and skin, and they are considered to be potential sensitizers.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation.

Label statements (Potential Sensitizer, may cause sensitization) and risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal. The label will restrict re-entry into treated areas until spray has dried.

7.3 Environmental Risk

The additional information provided to validate the viability of the test substance used in the non-target organism testing originally reviewed under ERC2010-01, *Metarhizium anisopliae* strain F52 adequately addressed the concern that pathogenicity could not be previously assessed. It was determined that test material was viable at the time of testing and that *Metarhizium anisopliae* strain F52 is not pathogenic to the non-target organisms tested. The data are of high quality and were determined to be sufficiently complete. The use of Met52 EC Bioinsecticide containing *Metarhizium anisopliae* strain F52 is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to assess the risk of Met52

EC Bioinsecticide used as a commercial-class biological insecticide to suppress ticks and chinch bug in turf and reduce the number of thrips and whitefly in greenhouses.

As a specific precaution, the Met52 EC Bioinsecticide label includes standard environmental precautionary statements to prevent the contamination of aquatic habitats.

7.4 Value

Met52 EC Biopesticide (active ingredient is *Metarhizium anisopliae* strain F52) is a biological insecticide for use on foliage of labelled greenhouse food crops against whiteflies and thrips and on turf against hairy chinch bugs and ticks. *Metarhizium anisopliae* strain F52 may contribute to resistance management in integrated pest management programs for these pests. Few active ingredients are registered against ticks on turf.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Metarhizium anisopliae* strain F52 and Met52 EC Bioinsecticide containing the technical grade active ingredient *Metarhizium anisopliae* strain F52, to reduce the numbers of whiteflies and thrips on greenhouse food crops and suppress hairy chinch bugs and ticks on turf.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

♀	female
♂	male
µg	micrograms
ARSEF	Agricultural Research Service Entomopathogenic Fungi
ATCC	American Type Culture Collection
bw	body weight
CFU	colony forming unit
EC	emulsifiable concentrate
g	gram
h	hour(s)
ha	hectare(s)
HE	high efficiency
IMI	Centre for Agriculture and Biosciences International Fungal Reference Collection
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	level of concern
LOD	limit of detection
m	metre(s)
MPCA	microbial pest control agent
mL	millilitre
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
PMRA	Pest Management Regulatory Agency
rDNA	ribosomal deoxyribonucleic acid
RQ	risk quotient
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
US	United States
w/v	weight per volume

Appendix I Tables and Figures

Table 1.0: Dermal Toxicity and Irritation of the End-use Product, Met52 EC Bioinsecticide

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Dermal Toxicity and Dermal Irritation of Met52 EC Bioinsecticide				
Acute Dermal Toxicity	Rabbit- New Zealand White; 5/sex, 5 g/kg bodyweight (bw) for 24 hours; daily clinical observations; sacrifice and gross necropsy on Day 15	LD_{50} > 5 g/kg bw	There were no mortalities or treatment-related effects on bodyweight/bodyweight gain All rabbits had signs of erythema and/or edema; symptoms resolved in all rabbits before sacrifice No treatment related necropsy findings. **LOW TOXICITY ACCEPTABLE**	PMRA 2143929
Dermal Irritation	Rabbit- New Zealand White; 2♂ and 1♀; 0.5 mL for 4 hours, observed for irritation for 7 days	Maximum irritation score of 1.33/8 after 72 hours	Very slight to well-defined erythema and edema was observed in 2 male rabbits. Superficial flaking of the test site skin was observed in 2 male rabbits on Day 7, otherwise, he female rabbit had completely recovered from signs of dermal irritation by Day 7. **SLIGHTLY IRRITATING TO SKIN** **ACCEPTABLE**	PMRA 2143929

Table 2 Alternative Active Ingredients Registered for Use on Supported Met52 EC Bioinsecticide Crop/Pest Combinations

Crop	Pest	Resistance Management Group: Active Ingredient(s)
Greenhouse Tomato	Whiteflies	Unclassified: Insecticidal Soap, *Beauveria bassiana* strain GHA 1B: Dichlorvos, Malathion, Naled 3A: Permethrin, Pyrethrins 4A: Acetamiprid, Imidacloprid 7C: Pyriproxyfen 23: Spiromesifen, Spirotetramat
Greenhouse Pepper, Greenhouse Strawberry, Greenhouse Zucchini	Thrips	Unclassified: *Beauveria bassiana* strain GHA 1B: Diazinon 3A: Pyrethrins 5: Spinetoram, Spinosad
Turf	Hairy chinch bug	Unclassified: Insecticidal Soap 1A: Carbaryl 1B: Chlorpyrifos 3A: Deltamethrin, Pyrethrins 4A: Clothianidin
	Ticks	Unclassified: Insecticidal Soap 1A: Propoxur 3A: Permethrin, Pyrethrins

Table 3 Use Claims Proposed by Applicant and Whether Acceptable or Unsupported

Claims proposed by applicant	Acceptable claims	Unsupported claim
CROP: Greenhouse tomato **PESTS:** Whiteflies (*Trialeurodes* sp. & *Bremesia* sp.) – control **RATE:** 0.5-5.0 L/1000 L water/ha **APPLICATION INTERVAL:** 5–10 days	**CROP:** Greenhouse tomato **PESTS:** Reduces numbers of whiteflies **CONCENTRATION:** 0.5-5.0 L/1000 L **APPLICATION INTERVAL:** Determined by monitoring, 5–10 days recommended	
CROPS: Greenhouse pepper, greenhouse strawberry, greenhouse zucchini **PESTS:** Thrips - control **RATE:** 0.5-5.0 L/1000 L water/ha **APPLICATION INTERVAL:** 5–10 days	**CROPS:** Greenhouse pepper, greenhouse strawberry, greenhouse zucchini **PESTS:** Reduces numbers of thrips **CONCENTRATION:** 0.5-5.0 L/1000 L **APPLICATION INTERVAL:** Determined by monitoring, 5–10 days recommended	
CROP: Turf **PESTS:** Chinch bug - control **RATE:** 64-96 mL/100 m^2 **APPLICATION INTERVAL:** 4–6 weeks	**CROPS:** Turf **PESTS:** Suppresses hairy chinch bug **RATE:** 64–96 mL/100 m^2 **APPLICATION INTERVAL:** Determined by monitoring, 4 weeks recommended	

Claims proposed by applicant	Acceptable claims	Unsupported claim
CROP: Turf **PESTS:** Ticks - control **RATE:** 137-155 mL/100 m^2 **APPLICATION INTERVAL:** 4–6 weeks	**CROPS:** Turf **PESTS:** Suppresses ticks **RATE:** 137–155 mL/100 m^2 **APPLICATION INTERVAL:** Determined by monitoring, 3 weeks recommended	
APPLICATION INSTRUCTIONS: Apply using hand-held, ground and/or aerial spray equipment. Do not apply through a thermal pulse fogger. Do not mix Met52 EC Bioinsecticide with fungicides. Make the first Met52 EC Bioinsecticide application against low population levels. Under high pest pressure use the higher rate.	**APPLICATION INSTRUCTIONS:** Apply using ground application equipment. Do not apply through a thermal pulse fogger. Do not mix Met52 EC Bioinsecticide with fungicides. Do not apply fungicides near the time of application of Met52 EC Bioinsecticide. Make the first Met52 EC Bioinsecticide application against low population levels. Under high pest pressure use the higher rate.	Aerial application.

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

PMRA No.	Reference
2143911	2011, Product Characterization and Analysis, DACO: M2.1,M2.2,M2.3,M2.4, M2.5,M2.6 CBI
2143912	2011, Characterization of the MCPA, DACO: M2.7.1, M2.7.2 CBI
2143913	2007, Genetic Diversity of *Metarhizium anisopliae* var. *anisopliae* in Southwestern British Columbia, DACO: M2.10.1, M2.7.2
2143914	2007, Review on the safety of the entomopathogenic fungus *Metarhizium anisopliae*, DACO: M2.7.2
2143915	1999, Measurement of fungal toxins destruxin and cytochalasin in *Metarhizium anisopliae* growth, DACO: M2.7.2 CBI
2143916	2011, Description of Beginning Materials Manufacturing Process for Met52 EC, DACO: M2.8 CBI
2143917	2011, Batch Analysis for Met52 EC, DACO: M2.10.1,M2.10.2,M2.8,M2.9.2, M2.9.3 CBI
2143918	2011, Disclosure of Ingredients, DACO: M2.9.1, M2.9.2, M2.9.3 CBI
2143923	2011, Request for MRL Exemption for Met52 EC Bioinsecticide, DACO: M2.9.3 CBI
2143924	2011, Toxicity/Pathogenicity Testing of *Metarhizium anisopliae,* Strain F52, Following Acute Oral Challenge in Rats, DACO: M2.9.3 CBI
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2.0 Human Health

PMRA No.	Reference
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2143929	2002, PMRA_Met52EC_EP_M4.4, DACO: M4.4
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2143931	2002, PMRA_Met52EC_EP_M4.5.2, DACO: M4.5.2
2143932	2011, PMRA_Met52EC_EP_M4.6, DACO: M4.6

3.0 Environment

PMRA No.	Reference
2210984	1998, DACO_M9.5.1_CoA Lot 980820., DACO: M9.5.1
2210986	1998, DACO_M9.5.1_CoA Lot 980923, DACO: M9.5.1

4.0 Value

PMRA No.	Reference
2143933	2011, PMRA_Met52EC_EP_M10.1, DACO: M10.1
2143934	2011, PMRA_Met52EC_EP_M10.2.2b, DACO: M10.2.2b
2143935	2011, PMRA_Met52EC_EP_M10.2.2c, DACO: M10.2.2c
2143936	2011, PMRA_Met52EC_EP_M10.2.2d, DACO: M10.2.2d
2143937	2011, PMRA_Met52EC_EP_M10.2.2e, DACO: M10.2.2e
2143938	2011, PMRA_Met52EC_EP_M10.2.2f, DACO: M10.2.2f
2143939	2011, PMRA_Met52EC_EP_M10.2.2g, DACO: M10.2.2g
2143940	2011, PMRA_Met52EC_EP_M10.2.2h, DACO: M10.2.2h
2143941	2011, PMRA_Met52EC_EP_M10.2.2i, DACO: M10.2.2i
2143942	2011, PMRA_Met52EC_EP_M10.2.2j, DACO: M10.2.2j
2143943	2011, PMRA_Met52EC_EP_M10.2.2k, DACO: M10.2.2k
2143944	2011, PMRA_Met52EC_EP_M10.2.2l, DACO: M10.2.2l
2143945	2011, PMRA_Met52EC_EP_M10.2.2m, DACO: M10.2.2m
2143946	2011, PMRA_Met52EC_EP_M10.2.2n, DACO: M10.2.2n
2143947	2011, PMRA_Met52EC_EP_M10.2.2o, DACO: M10.2.2o

2143948 2011, PMRA_Met52EC_EP_M10.2.2p, DACO: M10.2.2p

2143949 2011, PMRA_Met52EC_EP_M10.2.2q, DACO: M10.2.2q

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2143951 2011, PMRA_Met52EC_EP_M10.2.2s, DACO: M10.2.2s

2143952 2011, PMRA_Met52EC_EP_M10.2.2t, DACO: M10.2.2t

2143953 2011, PMRA_Met52EC_EP_M10.2.2u, DACO: M10.2.2u

2143954 2011, PMRA_Met52EC_EP_M10.3_M10.4, DACO: M10.3, 10.4

2143955 2011, PMRA_Met52EC_EP_M12.5.2_M12.5.4, DACO: M12.5.2, 12.5.4

2143956 2011, PMRA_Met52EC_EP_M12.5.2_M12.5.4b, DACO: M12.5.2, 12.5.4b

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2212438 2012, Response to clarification e-mail, DACO: 0.8